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# EARLY EFFECTS OF TRIMETHYLTIN ON THE DENTATE GYRUS BASKET CELLS: A MORPHOLOGICAL STUDY

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*Electrophysiological evidence for reduction of recurrent inhibition in the dentate gyrus in animals exposed to trimethyltin (TMT) suggested alterations in the inhibitory neurons (basket cells) by TMT. The present study was designed to investigate the morphology of basket cells after TMT exposure. Long-Evans hooded rats were injected with TMT chloride in a dose of 6.0 mg/kg body weight (b.w.). Tissue samples from the dentate gyri were examined by both light and electron microscopy at 24 and 72 h after TMT exposure. Except for isolated basket cell damage at 72 h, no remarkable pathological changes were observed with light microscopy. Consistent with previous data, electron microscopy revealed that the basket cells of the dentate gyrus are large neurons situated just below the granule cell layer with characteristic large, infolded nuclei and intranuclear filamentous rods. Increased cytoplasmic density and degenerative changes of the Golgi complex were evident in the basket cells as early as 24 h after TMT exposure. By 72 h, neuronal vacuolation, accumulation of lysosomes, and occasional neuronal necrosis were observed. No significant pathological changes were found among the granule cells at this time. This report provides the first morphological evidence for early damage to the basket cells by TMT, which may account for the reduction of recurrent inhibition and hyperexcitability among the granule cells reported previously.*

## INTRODUCTION

Neuropathological studies with rats have revealed damage to the pyramidal neurons in Ammon's horn after exposure to trimethyltin

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(TMT) (Chang and Dyer, 1983, 1984). The pattern of hippocampal damage induced by TMT is reminiscent of damage described by others following excessive stimulation or excitation in the mossy fiber system (Sloviter and Damiano, 1981a,b; Sloviter, 1984). Among potential mechanisms by which excessive stimulation of $CA_3$ pyramidal neurons may occur is the reduced inhibitory control of the granule cells, whose mossy fiber axons form excitatory synapses with dendrites of $CA_3$ neurons. Two lines of evidence support the hypothesis that reduced inhibition is one of the consequences of exposure to TMT. First, electrophysiological data suggest a reduction in the recurrent inhibition of granule cells in the dentate gyrus (Dyer et al., 1982; Dyer and Boyes, 1984). Second, biochemical studies by Doctor et al. (1982) and by Valdes et al. (1983) revealed a TMT-induced disruption in GABA metabolism, both in vivo and in vitro.

The disinhibition-induced excessive stimulation hypothesis for TMT-induced pyramidal cell damage would be further strengthened by morphological evidence for dysfunction in inhibitory neurons. In the dentate gyrus, it is generally assumed that basket cells provide a major source of inhibition onto granule cells. Ramón y Cajal (1911) and Lorente de Nó (1934) gave the earliest descriptions of dentate gyrus basket cells. Most of these cells have a pyramidal-shaped soma that lies either embedded in the granule-cell layer or directly beneath it. A single or branching apical dendrite ascends through the granule cell layer, and basal dendrites run parallel to this layer or flow into the region below it. The axons of these pyramidal-shaped basket cells usually arise from an apical dendrite and arborize within the granule-cell layer to form a basket plexus (Gayoso et al., 1979).

Ribak and Seress (1983) described five different types of basket cells in the rat dentate gyrus. Although these basket cells differ slightly in their shapes and dendritic or axonal arborizations, they are believed to have similar basic functions. Glutamic acid decarboxylase, the enzyme responsible for synthesis of GABA, has been localized within somata as well as axon terminals of all types of basket cells (Barber and Saito, 1976; Ribak et al., 1978; Seress and Ribak, 1983), supporting the contention that they are GABAergic. Electron microscopic studies of basket cells have revealed infolded nuclei, intranuclear rods or sheets, and an abundance of organelles in the perikaryal cytoplasm of these cells. Therefore basket cells are different from granule cells (Seress, 1978; Ribak and Anderson, 1980; Ribak and Seress, 1983), but similar to many other GABAergic neurons in the cerebral cortex (Ribak, 1978; Ribak et al., 1978).

The fact that mossy-fiber collaterals synapse with basket cells, which in turn synapse onto the somata or proximal dendrites of the granule cells (Ribak and Seress, 1983), suggests that the basket cells provide recurrent inhibition of the granule cells (Andersen et al., 1966; Barber

and Saito, 1976; Ribak et al., 1978; Seress and Ribak, 1983). Recent studies have further suggested that basket cells may mediate a feed-forward inhibition in the dentate gyrus, whereby excitation of commissural axons may cause basket-cell activation that inhibits granule cells (Buzsáki and Eidelberg, 1981; Douglas et al., 1983; Seress and Ribak, 1984).

The present study was designed to search for evidence of damage to the basket cells in the dentate gyrus following exposure to TMT. Emphasis was placed on time points relatively close to treatment for early changes in these cells.

## METHODS

Young adult male Long-Evans hooded rats (60-80 d old) were used in the experiment. Animals were exposed to trimethyltin chloride via oral intubation in a dose of 6.0 mg TMT/kg body weight (b.w.) (or 7.5 mg TMT-Cl/kg b.w.). Animals were then sacrificed in groups of 5 animals at 24 and 72 h following exposure. The saline vehicle was administered to the control animals, which were sacrificed similarly to the experimental animals.

At sacrifice, animals were anesthetized and perfused intracardially with saline solution followed by 2.5% buffered glutaraldehyde. Brains were then removed surgically and further immersion-fixed in a solution of 10% buffered formalin for at least 48 h before tissue processing. After dehydration in graded ethanols and embedding in Paraplast, brains were sectioned at 6 $\mu$m and stained with hematoxylin-eosin (H&E) for general histopathological examination.

For electron microscopy, the hippocampal formation was carefully dissected away from other brain structures and fixed in 2.5% buffered glutaraldehyde and embedded with Epon. Semithin (1.0 $\mu$m) sections were made to locate the basket cells before ultrathin sections were made for electron microscopic examination. Criteria for their identification were those specified by others (Seress, 1978; Ribak and Anderson, 1980; Ribak and Seress, 1983), particularly large size, invaginated nuclei, and intranuclear filamentous rods or sheets.

## RESULTS

Light-microscopic survey revealed fusiform or pyramidal-shaped neurons located just beneath the inner borders of the dentate granule cell layers (Fig. 1). Except for occasional necrotic changes at 72 h (Fig. 2), light microscopy did not reveal any remarkable pathology in these putative basket cells.

Electron microscopy confirmed the presence of large neurons fulfilling the criteria for basket cells with characteristic infolded nucleus

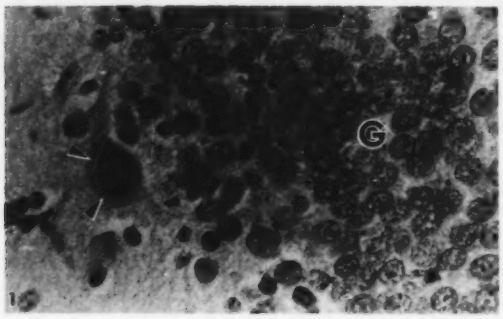


**FIGURE 1.** Dentate gyrus, rat, control. The basket cell (arrowhead) appeared to be a large pyramidal or fusiform neuron located just below the granule cell layer (G). ×315.

and intranuclear filamentous rods or sheets (Figs. 3 and 4). The basket cells also displayed well-developed cytoplasmic organelles, including mitochondria, Golgi complex, and rough endoplasmic reticulum.

At 24 h after TMT exposure, a dense cytoplasmic appearance was observed in many basket cells of all the animals studied. Careful examination demonstrated an increase in both rough endoplasmic reticulum and ribosomal clusters in these neurons (Fig. 5), yielding a dense cytoplasmic appearance. A careful survey also revealed degenerative changes of the Golgi complex giving rise to cytoplasmic membranous whorls (Figs. 6, 7, and 8) in these neurons. Extensive dilatation and vacuolation of the Golgi complex (Figs. 9 and 10) were also observed, leading to multiple cytoplasmic vacuolations of many basket cells (Fig. 11). Similar vacuolar changes in many of the interneurons within the hilar area were also found (Fig. 12).

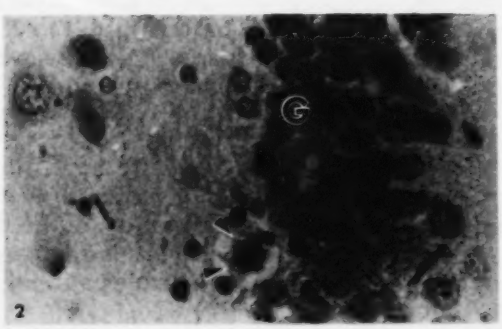


**FIGURE 2.** Dentate gyrus, TMT-treated, 72 h. Careful survey revealed isolated necrosis of the basket cells (arrowheads), while the granule cells (G) still appeared intact. ×315.

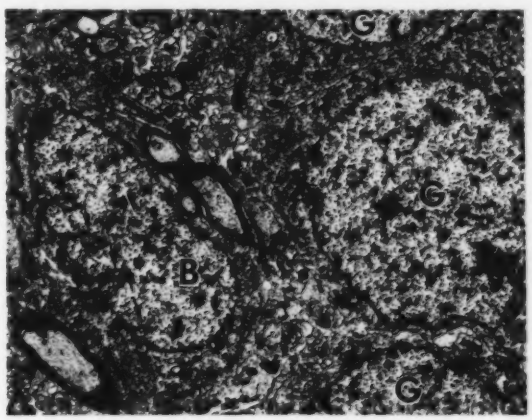


**FIGURE 3.** Dentate gyrus, rat, control. A basket cell (B) located just deep to the granule cell (G) layer, showing the characteristic intranuclear filamentous rod or sheet (arrowhead) and infolded (arrows) nucleus. ×4500.

Vacuolar changes could still be found in the basket cells 72 h following TMT intoxication (Fig. 13). At this time of intoxication, many basket cells also displayed an accumulation of electron-dense bodies, presumably lysosomes, in their cytoplasm (Figs. 14 and 15). Necrotic changes of the basket cells (Fig. 16), although only an occasional finding, could be demonstrated. In sum, early degenerative and necrotic changes in the dentate basket cells were observed following TMT exposure, suggesting extreme vulnerability of these inhibitory neurons to TMT toxicity.

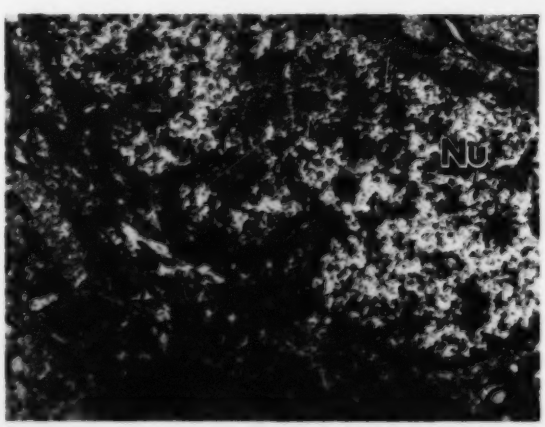


**FIGURE 4.** An enlargement of the basket cell from Fig. 3; showing intranuclear filamentous rod or sheet (arrowhead) and infolded nucleus (arrows). Nu, nucleus. The cytoplasm is also abundant with organelles. ×6300.

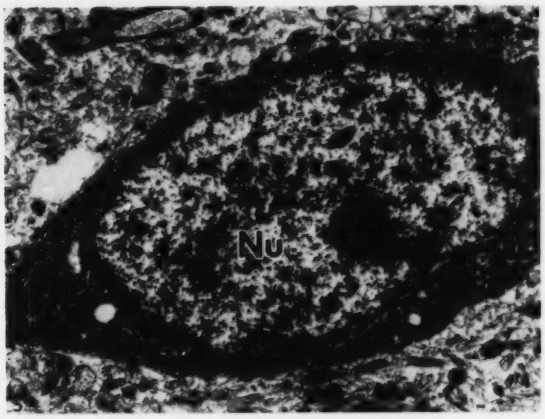


**FIGURE 5.** Basket cell, TMT-treated, 24 h. An increase in cytoplasmic density, presumably due to an increase in ribosomal clusters, was observed. Nu, nucleus; arrow, nuclear infolding; arrowhead, intranuclear filamentous rod. ×5040.

## DISCUSSION

The present study provides the first morphological evidence of early toxic damage to the basket cells of the dentate gyrus. Since these cells mediate GABAergic inhibition and have reciprocal connections with granule cells (Ribak and Seress, 1983), this observation is consistent with the electrophysiological data indicating an early loss of recurrent inhibition among the dentate granule cells after TMT intoxication (Dyer et al.,

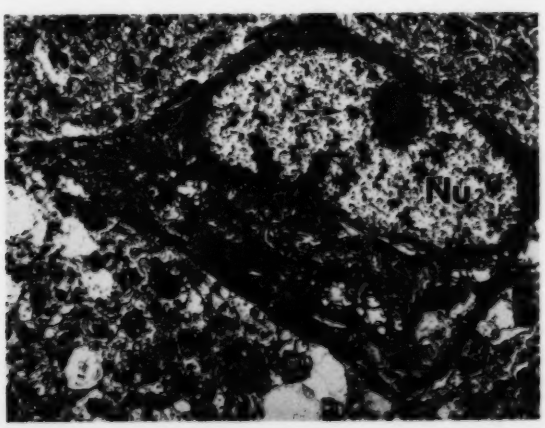


**FIGURE 6.** Basket cell, TMT-treated, 24 h. Degenerative changes of cytoplasmic membranes to form myelin-figures (arrowhead) were observed. Nu, nucleus; arrow, intranuclear filamentous rod. ×5040.

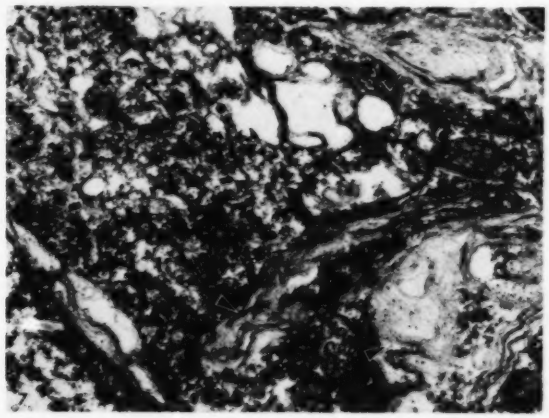

**FIGURE 7.** Higher magnification of Fig. 6 to show the myelin-figure formation (arrowhead) in a basket cell cytoplasm after 24 h exposure to TMT. ×10,150.

1982; Dyer and Boyes, 1984). It is important to note that significant morphological changes in the basket cells occurred within 24 h following TMT exposure, a time at which no remarkable behavioral changes and pathological lesions have been found in either the granule cells or pyramidal neurons at the dose used here (Chang and Dyer, 1983). While the nature of the morphological changes (vacuolation, lysosomal accumulation, necrosis) induced by TMT in the basket cells was nonspecific, the early impact of TMT on basket-cell neurons provides clues to

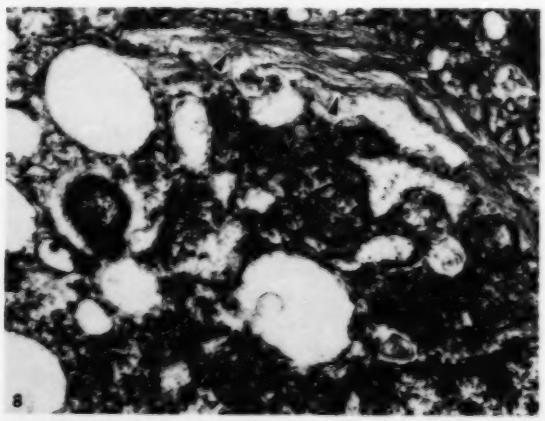

**FIGURE 8.** Basket cell, TMT-treated, 24 h. Careful examination revealed degenerative changes of the Golgi complex (arrowheads), which probably gave rise to the cytoplasmic myelin-figures. ×10,150.

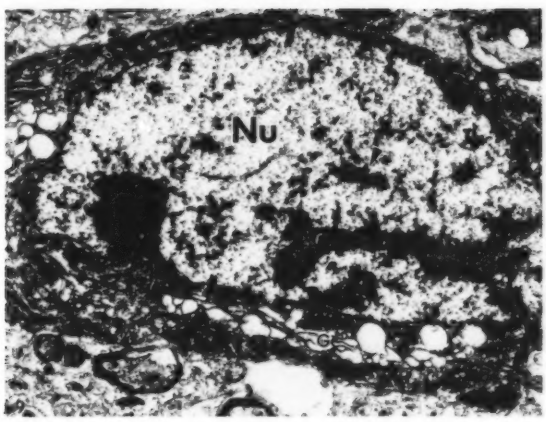


**FIGURE 9.** Basket cell, TMT-treated, 24 h. Increase in cytoplasmic matrical density with vacuolar degenerative changes of the Golgi compex (G) typified the early changes of the basket cells. Nu, nucleus; arrowhead, intranuclear filamentous rod. ×5040.

the pathogenic mechanisms underlying the eventual necrosis of $CA_3$ neurons in the hippocampal formation. Although many of the cellular changes among the basket cells are potentially reversible, these changes represent cell injuries and would affect the normal functions of these cells.

Quantitative assessment (Seress and Pokorny, 1981) indicates that the basket cell/granule cell ratio in the dentate gyrus is approximately 1:200.

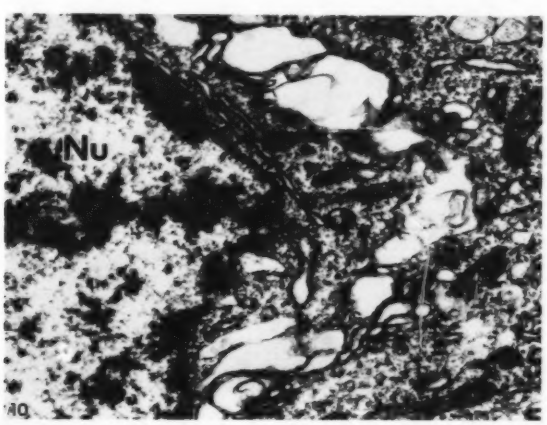


**FIGURE 10.** Enlargement of a basket cell showing vacuolar changes of the Golgi complex (G) after 24 h exposure to TMT. Nu, nucleus. ×10,150.

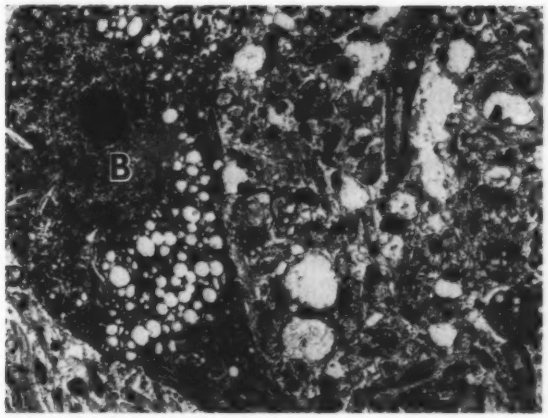


**FIGURE 11.** Dentate gyrus, TMT-treated, 24 h. Extensive vacuolation of a basket cell (B) was demonstrated. ×3290.

Although there are many more granule cells than basket cells in the dentate gyrus, the widely distributed basket-cell axons suggest far reaching and potent inhibitory control. Consequently, loss of a relatively small number of basket cells would mean a loss of inhibitory influence over a vast number of granule cells and set the stage for an excess granule cell output.

Although the precise function of the large polymorphic (pyramidal)

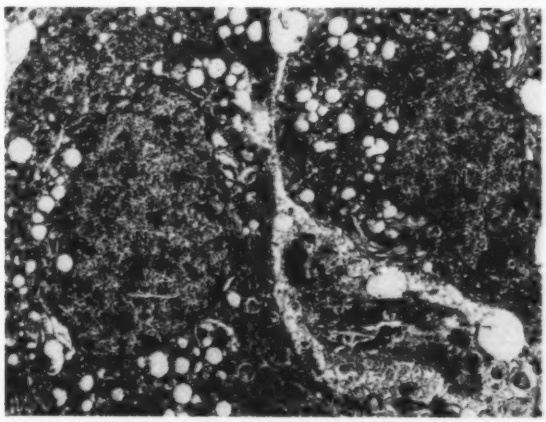

**FIGURE 12.** Hilus area (field $CA_4$), TMT-treated, 24 h. Two $CA_4$ interneurons (N) also showed similar vacuolar changes as those observed in the basket cells. The $CA_4$ interneurons (polymorphic neurons) are believed to have physiological functions similar to those of the basket cells. ×3290.

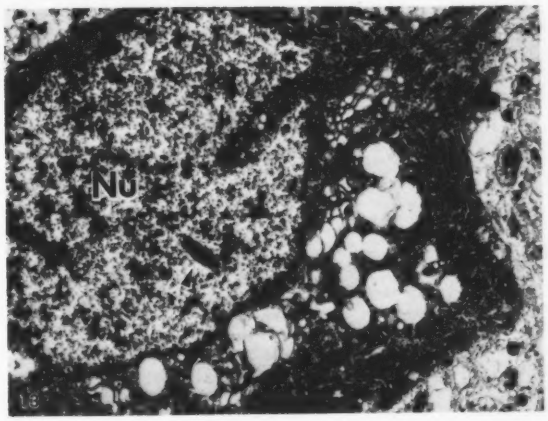


**FIGURE 13.** Basket cells, TMT-treated, 72 h. Some basket cells still showed an increased cytoplasmic density and vacuolar changes of the Golgi complex 72 h after TMT exposure. Nu, nucleus; arrowhead, intranuclear filamentous rod. ×5950.

neurons in the hilus ($CA_4$) area is still obscure, they are believed to have GABAergic inhibitory function similar to that of the basket cells (Amaral, 1978; Seress and Ribak, 1983). Our present study also shows early damage in these interneurons, which further suggests the selective toxic action of TMT on the GABAergic inhibitory cells in this region of the nervous system.

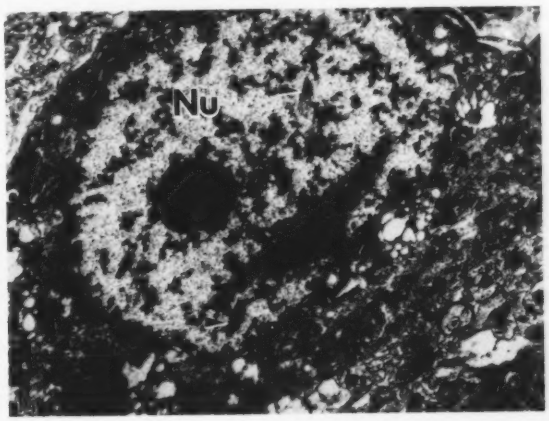


**FIGURE 14.** Basket cell, TMT-treated, 72 h. By this time, many basket cells displayed an increase in electron-dense bodies, presumably lysosomes, in the cytoplasm. Nu, nucleus; arrowhead, intranuclear filamentous rod. ×5040.

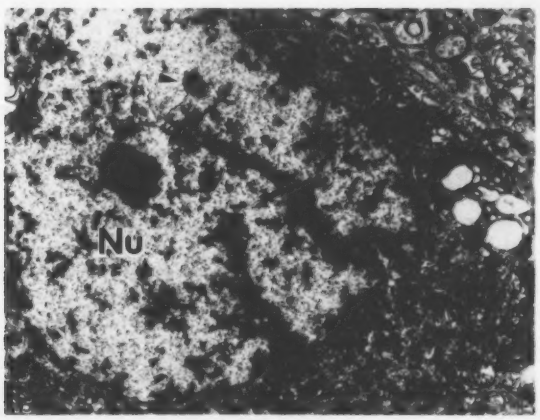


**FIGURE 15.** Basket cell, TMT-treated, 72 h. Large number of cytoplasmic dense bodies, presumably lysosomes, was observed within the basket cells. Nu, nucleus; arrowhead, intranuclear filamentous rod. ×5040.

In summary, the present study indicates that alterations occur in dentate gyrus basket cells shortly after administration of TMT. This finding supports the hypothesis that eventual destruction of $CA_3$ neurons by TMT may result from overactivation from granule cells that have lost inhibitory input.

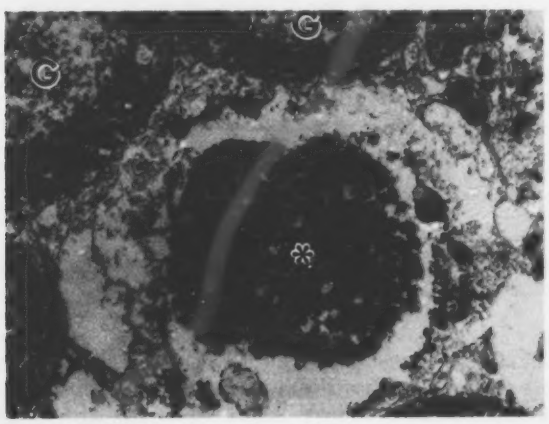


**FIGURE 16.** Dentate gyrus, TMT-treated, 72 h. A large necrotic cell (asterisk), presumably a basket cell, was found just below the granule cell layer. The granule cells (G) still showed no remarkable changes at this time. ×5950.

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